Trends and changes in *Clostridium difficile* diagnostic policies and their impact on the proportion of positive samples: a national survey

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Abstract

In June 2012, Israeli guidelines for laboratories were published defining the recommended methods for diagnosis of *Clostridium difficile* infection (CDI). We conducted this survey to examine the effects of the new recommendations on the proportions of rejected and positive samples by the different methods. A survey was mailed to the directors of all general hospital (GH) and health maintenance organization (HMO) clinical microbiology laboratories. The report was divided into two periods, before and after implementation of the guidelines. Surveys were completed by 13/28 GH laboratories and 5/6 HMO laboratories. All 18 of these laboratories used *C. difficile* toxin (CDT) enzyme immunoassay alone during the first period of the survey. In the second period, nine laboratories (Group A) used CDT-PCR: two of them used this method exclusively while the other seven used it to resolve most (>90%) of the discrepant results (glutamate dehydrogenase antigen (GDH) +/CDT−]. The other nine laboratories (Group B) used combined GDH/CDT assay, using CDT PCR in only a minority (< 20%) of GDH+/CDT− cases. The overall proportion of rejected samples increased from 9.5% in the first period to 13.9% in the second ($p < 0.001$). Between the first and second periods the proportion of positive samples increased from 9.0% to 11.6% in group A laboratories ($p < 0.001$), but decreased from 12.9% to 9.7% in group B laboratories ($p < 0.001$). Implementation of the guidelines has resulted in a significant increase in the proportion of rejected samples and in the proportion testing positive, suggesting more appropriate test utilization and improved sensitivity in the laboratory diagnosis of CDI.

Keywords: *Clostridium difficile*, glutamate dehydrogenase antigen, guidelines, national, polymerase chain reaction, pre-analytical screening
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Introduction

Clostridium difficile is one of the leading causes of healthcare-associated infection [1] and may be the most common cause of bacterial gastroenteritis in developed countries [2]. Since the beginning of the millennium, there has been an increase in the incidence of *Clostridium difficile* infection (CDI) and possibly also in the severity of illness caused by it in certain parts of the USA and Canada [1,3]. These changes are attributed in part to the spread of an epidemic clone, BI/NAP1/027, that has since been identified also in Europe, East Asia and Australia [1].

Concomitant with these epidemiological changes, there have been dramatic changes in the methodology available for the laboratory diagnosis of CDI. Methods such as toxigenic culture or cell culture cytotoxicity neutralization assay that have traditionally been used by reference laboratories are still considered the gold standard [4]. However, most clinical laboratories have been using enzyme immunoassay (EIA) for the *C. difficile* toxins A and B (CDT EIA) as their routine diagnostic method [2]. The accumulation of data from numerous studies has shown variable and frequently low sensitivity

values ranging from 43% to 98% [4]. The specificity of these tests appears to be higher. However, the positive predictive values are as low as 50% in some studies [4]. Therefore, several recent guidelines [5,6], although not all [7], have stipulated that CDT EIA cannot be used as a sole method for diagnosis of CDI. Instead, other methods, including CDT PCR and glutamate dehydrogenase (GDH) are recommended, the latter requiring confirmation of toxin production by an additional method. Despite these updates in the guidelines, several national [8–10] and international [11] surveys have found that these newer methods are used by only a minority of laboratories, highlighting the potential for under-diagnosis of CDI.

The diagnosis of CDI requires the presence of both relevant clinical symptoms as well as laboratory confirmation, because toxigenic *C. difficile* can be detected in asymptomatic individuals [5]. Therefore, professional guidelines [5–7] have recommended the application of sample selection criteria, such as the avoidance of testing repeat samples or samples taken from young infants. The extent to which such criteria are applied can have a significant influence on the proportion of samples testing positive. This effect can be even more pronounced with the application of the newer, more sensitive diagnostic methods. However, the use of rejection criteria has been addressed by only one [8] of the national [9,10] and international [8,11] surveys conducted, and none of these evaluated the effect of the changing diagnostic methodology on the proportion testing positive.

In Israel, official guidelines were issued by the Ministry of Health in June 2012 [12]. Following the publication of these guidelines, we conducted a national survey that aimed to (i) examine the methods and policies regarding CDI diagnosis in Israel, (ii) study the policies regarding sample selection and the proportions of rejected samples, and (iii) assess the proportion of positive samples by the different methods, before and after implementation of the newer diagnostic methods.

Methods

Study design and questionnaire

This was a nationwide survey of clinical microbiology laboratories, regarding the practices of CDI microbiological diagnosis. The survey included a written questionnaire sent to the directors of participating laboratories in August 2012. Participating laboratories included those of all general hospitals (GH) as well as those of the Health Maintenance Organizations (HMO). The latter serve both community patients and affiliated long-term care facilities (LTCF). Three laboratories located in LTCF and one located in a private hospital (<200 beds) were not approached. In the survey the directors were asked to provide data regarding the current method of CDI diagnosis (type of test and manufacturer), the duration of its use in the facility, and information regarding the previously used method. They were also asked to indicate whether they apply criteria for sample acceptance and if so, since when. All participating laboratories provided monthly data regarding the total number of CDI tests, the number of rejected samples, the number of negative tests and the number of positive tests. They were asked to provide data regarding the methodology currently in use as well as that used previously. Data were collected mainly retrospectively: laboratories were asked to submit data for up to 12 months previously, but were also allowed to collect and submit data prospectively from survey initiation (August 2012) until December 2012.

Official guidelines for the diagnosis of CDI in Israel

The first official guidelines regarding the diagnosis of CDI in Israel were issued by the Ministry of Health in 2000. Clinical laboratories were instructed to use EIA that test for both toxins A and B [13]. In June 2012, a new guidelines document was issued [12]. In these guidelines, instructions regarding both sample acceptance criteria and analytical procedures were given. Criteria for sample rejection were given in accordance with the Infectious Diseases Society of America guidelines [5], including: (i) rejection of solid stool samples (with the exception of cases in which ileus is the presenting symptom), (ii) non-repetition of testing within 2 weeks of a positive test or 1 week of a negative test, and (iii) rejection of samples from neonates. The analytical procedures recommended offered two options: (i) screening using GDH antigen testing, with confirmation of the presence of CDT in positive samples by CDT EIA or CDT PCR, with PCR recommended in the event of discrepant results by GDH and EIA testing; (ii) CDT PCR as a single test. The guidelines did not include specific recommendations for the indications for CDI testing, which was done per request only.

Definitions

Participating laboratories were classified according to institution type and the diagnostic method in use at the time of the survey (second period).

Institutions. Laboratory classification according to institution type was as follows: group 1, tertiary-care hospital; group 2, non-tertiary-care GH, >400 beds; group 3, GH, <400 beds; group 4, HMO laboratories. The HMO laboratories varied widely in the volume of samples handled; all serve outpatient clinics as well as LTCFs.

Diagnostic methods. Laboratory classification according to diagnostic method was as follows: group A, use of CDT PCR exclusively or in most cases (>90%) of GDH+/CDT− samples;

group B, no use of CDT PCR or use in only a minority (<20%) of GDH+/CDT− samples. Both groups were using CDT EIA during the first period. Hence, the time periods were defined individually for each laboratory, according to the time of transition between the methods. We included only laboratories already using GDH/CDT and/or CDT PCR and able to provide results for a span of between 6 and 12 months for each period. Although it provided data for only 3 months of the second period, we included laboratory no. 4 so as to include data also from the only laboratory that was using an in-house CDT PCR assay.

Data analysis

The total number of patients with CDI was defined as the total number of CDT EIA-positive samples plus the number of CDT PCR-positive/CDT EIA-negative samples. Sensitivity of CDT EIA was calculated as the number of CDT EIA-positive samples (true positives) divided by the total number of patients with CDI.

Categorical parameters were compared using the Pearson χ^2 test. Values of $p \leq 0.05$ were considered as representing a significant difference between the groups. Multivariable analysis was performed using binary logistic regression prediction models constructed via forward stepwise progression. Data were analysed using the SPSS software package version 15.0 (SPSS, Chicago, IL, USA).

Results

General characteristics and sample rejection policies of participating laboratories

Overall, 18 laboratories completed the survey. The selection process is presented in Fig. 1. Participating laboratories included those from four group one hospitals, seven group two hospitals and two group three hospitals, as well as ten HMO-based laboratories (Fig. 1, Table 1)—five covering 8286 (52%) of Israel's 15 937 acute-care hospital beds and five covering 91% of HMO-insured patients.

The transition between the two periods occurred in 2010 in three laboratories, 2011 in eight laboratories and 2012 in seven laboratories (Table 1). During the second period, two laboratories were using CDT PCR as their only method (Table 1) and the rest of the laboratories were using a combined GDH and CDT EIA test (GDH/CDT) as their primary method, almost all from a single manufacturer (C. DIFF QUIK CHEK COMPLETE®, TECHLAB, Blacksburg, VA, USA). The Xpert® *C. difficile* test (Cepheid, Sunnyvale, CA, USA) was used by the majority of the laboratories; exceptions were laboratory no. 4, which used an in-house assay, and labs no. 6, 12 and 18, which used the Illumigene *C. difficile* (Meridian Bioscience, Inc., Cincinnati, OH, USA) assay.

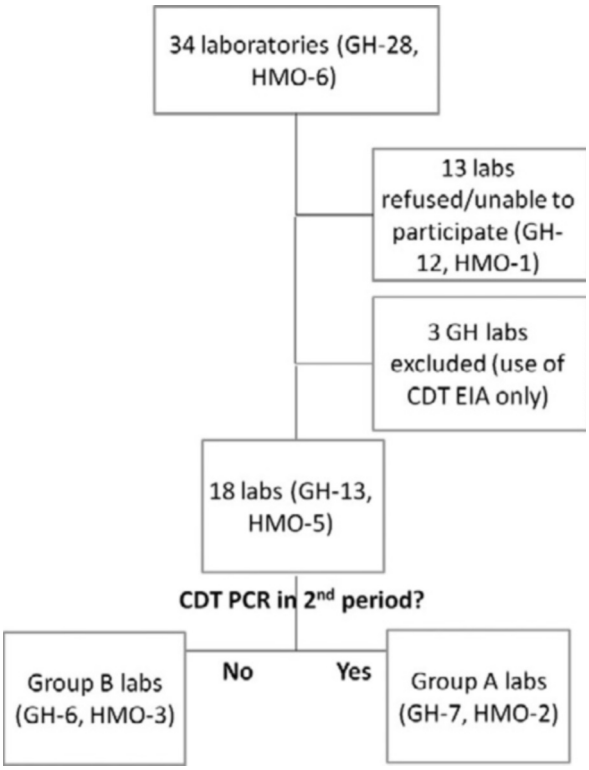

FIG. 1. Recruitment and classification of participating laboratories. GH, general hospital; HMO, health maintenance organization; CDT, *Clostridium difficile* toxin; GDH, glutamate dehydrogenase antigen. Laboratories were classified according to their use of CDT PCR in the second period as follows: group A, if they were using CDT PCR exclusively or in most cases (>90%) of GDH+/CDT− samples; group B, if they did not perform CDT PCR at all or in only a minority (<20%) of GDH+/CDT− samples.

Sample rejection/acceptance criteria were applied systematically by all except for two HMO-based laboratories (Table 1). The rejection criteria most commonly applied were recently diagnosed CDI (15/18 patient samples) and formed stool (14/18 patient samples). Overall, the proportion of patient samples rejected increased significantly from 9.5% in the first to 13.9% in the second period ($p < 0.001$, Table 2). The extent of this change was related to the application of rejection criteria (Table 1). In laboratories in which rejection criteria were used in both periods (laboratories nos. 2–4, 9, 12, 17, 18), the proportion of rejected samples increased from 9.9% to 11.9%, ($p < 0.001$) whereas in laboratories that applied them only in the second period (laboratories nos. 1, 5–8, 10, 11, 13, 16) this proportion increased from 9.5% to 19% ($p < 0.001$). Moreover, in the two laboratories that did not

TABLE 1. General characteristics of clinical laboratories participating in the survey

Lab. no.	Methods used in second period[a]	Trans date[b]	Reports, mon (1st/2nd)	Rejection criteria[c]	Criteria apply?[d]
Tertiary care hospitals					
1	GDH/CDT+PCR	03/12	11/10	2–5	2nd
2	GDH/CDT only	09/10	12/12	2,3,5	Both
3	GDH/CDT+PCR	08/11	13/12	2,3,5	Both
4	CDT PCR	05/12	16/3	2–5	Both
General hospitals, >400 beds					
5	GDH/CDT only	01/11	12/12	2,4	2nd
6	GDH/CDT+PCR	03/11	12/12	2,3	2-both, 3-2nd
7	CDT PCR	03/11	12/12	2–5	2,5-both, 3-4-2nd
8	GDH/CDT+PCR	07/12	12/8	2–5	2nd
9	GDH/CDT only	02/12	12/8	3–5	Both
10	GDH/CDT+PCR	05/12	8/8	2–5	2,5-both, 3-4-2nd
11	GDH/CDT only[e]	01/11	12/9	1,3,5	2nd
General hospitals, <400 beds					
12	GDH/CDT only[f]	04/11	12/12	1–5	Both
13	GDH/CDT only	09/10	12/12	2–3	2nd
Health maintenance organization laboratories					
14	GDH/CDT+PCR	07/12	6/8	None	NA
15	GDH/CDT only	12/11	12/12	None	NA
16	GDH/CDT+PCR	08/11	12/12	2–5	2-3,5-both, 4-2nd
17	GDH/CDT only	12/10	12/12	2,3,5	Both
18	GDH/CDT only	06/12	12/12	2–3	Both

[a]Methods used in the second period (first period-testing of *Clostridium difficile* toxin by EIA method): CDT PCR, *C. difficile* toxin (CDT) PCR; glutamate dehydrogenase antigen (GDH)/CDT+PCR, combined GDH/CDT testing, with CDT PCR performed for most cases (>90%) of GDH-positive, CDT-negative samples; GDH/CDT only, combined GDH/CDT testing, with CDT PCR performed for the minority of GDH-positive, CDT-negative samples.
[b]Trans date: the transition date between the periods (MM/YY).
[c]Rejection criteria: 1, requires infectious diseases specialist authorization; 2, rejection of formed stool samples; 3,4, rejection of samples from a recently positive- or negative-tested patient, respectively; 5, rejection of samples from infants.
[d]Implementation of rejection criteria during the two periods of the survey.
[e]CDT PCR was implemented for testing of all GDH-positive, CDT-negative samples in the last 3 months of the survey.
[f]GDH/CDT testing was used in the first 9 months of the second period, and CDT PCR was used for all samples in the following 3 months.

apply any criteria (nos. 14, 15), the proportion decreased from 3.5% to 1% ($p < 0.001$). The proportion did not change significantly in the HMO laboratories (nos. 14–18, p 0.199, Table 2). The proportion rejected was significantly higher ($p < 0.001$) in group A compared with group B laboratories in both periods. However, the inter-period proportion rejected increased in both groups, by 5.5% and 3.5%, in groups A and B, respectively.

Proportion positive among laboratories and periods

The proportions of positive samples were calculated based on reports of a positive CDT EIA in the two periods or positive CDT PCR in the second period (Table 2). The total proportion positive was 12.1% in the first period and did not change significantly in the second period. However, this proportion increased in group A from 8.7% to 12% ($p < 0.001$) but decreased in group B from 15.2% to 11.9% ($p < 0.001$). Accordingly, it was higher in group B than in group A in the first period (15.2% vs. 8.7%, respectively, $p < 0.001$) but was almost identical in both groups in the second period (p 0.87). The proportion positive increased significantly in five of nine group A laboratories and decreased significantly in four of nine group B laboratories, and was higher overall in HMO than in GH laboratories (Table 2). Of note, laboratory no. 13 had a higher number of positive isolates in the first period, probably due to lack of a sample rejection/acceptance policy, leading to repeat testing and reporting of positive samples (Table 1).

We constructed a multivariable analysis that included the diagnostic method, the period and the institution type. The proportion positive was significantly lower in GH compared with HMO laboratories: OR 0.726 (95% CI 0.664–0.795, $p < 0.001$) for GH group 1, OR 0.841(95% CI 0.764–0.926, $p < 0.001$) for GH group 2 and OR 0.438 (95% CI 0.309–0.621, $p < 0.001$) for GH group 3 versus HMO laboratories, respectively. The proportion positive was higher in the laboratories using the CDT PCR method (alone or combined with GDH) compared with that in laboratories using CDT EIA alone (OR 1.342, 95% CI 1.245–1.447, $p < 0.001$) or GDH/CDT combined testing without CDT PCR (OR 1.128, 95% CI 1.034–1.135, p 0.007).

Proportion of positive tests using CDT EIA, GDH antigen and CDT PCR during the second period of the survey

The proportion of positive samples using each of the three methods (CDT EIA, GDH antigen and CDT PCR) during the second period was compared (Table 3). We excluded from the analysis, laboratories that performed CDT PCR as their sole method (4 and 7) and laboratories that did not have detailed documentation of GDH results (3 and 11). The total proportions of GDH+/CDT+ samples and GDH+/CDT− samples were 10.3% and 7.8%, respectively. CDT PCR was performed in 9 of 14 laboratories, on a total of 608 of 1370 GDH+/CDT− samples (44%). It was positive in 334 of 608 samples (53%), with proportion positive in each laboratory ranging from 36% to 87%.

TABLE 2. Temporal trends in samples' rejection and positivity proportions

Lab.	Methods in second period	First period[a] Total	First period[a] Rejected (%)	First period[a] Pos. (%)[b]	Second period[a] Total	Second period[a] Rejected (%)	Second period[a] Pos. (%)[b]	Δ proportion rejected (P)	Δ proportion positive (P)
1	GDH/CDT+PCR	3350	557 (16.6)	211 (7.6)	3124	901 (28.8)	297 (13.4)	12.2 (<0.001)	5.8 (<0.001)
2	GDH/CDT only	3930	90 (2.3)	289 (7.5)	4383	196 (4.5)	236 (5.6)	2.2 (<0.001)	−1.9 (<0.001)
3	GDH/CDT+PCR	1863	263 (14.1)	193 (12.1)	2080	310 (14.9)	199 (11.2)	0.8 (0.484)	−0.9 (0.459)
4	CDT PCR	4420	369 (8.3)	336 (8.3)	1524	33 (2.1)	187 (12.5)	−6.2 (<0.001)	4.2 (<0.001)
5	GDH/CDT only	903	11 (1.2)	124 (13.9)	951	62 (6.5)	38 (4.3)	5.3 (<0.001)	−9.6 (<0.001)
6	GDH/CDT+PCR	1479	35 (2.4)	100 (6.9)	1514	35 (2.3)	167 (11.3)	−0.1 (0.921)	4.4 (<0.001)
7	CDT PCR	1302	206 (15.8)	89 (8.1)	1231	485 (39.4)	76 (10.1)	23.6 (<0.001)	2 (0.127)
8	GDH/CDT+PCR	396	43 (10.9)	30 (8.5)	234	29 (12.4)	26 (12.7)	1.5 (0.559)	4.3 (0.055)
9	GDH/CDT only	2057	31 (1.5)	220 (10.9)	1512	34 (2.2)	149 (10.1)	0.7 (0.102)	−1.2 (0.459)
10	GDH/CDT+PCR	571	72 (12.6)	29 (5.8)	701	225 (32.1)	80 (16.8)	29.5 (<0.001)	11 (<0.001)
11[c]	GDH/CDT only	1064	16 (1.5)	131 (12.5)	810	29 (3.6)	81 (10.3)	2.1 (<0.001)	−2.2 (0.16)
12[d]	GDH/CDT only	403	8 (2)	10 (2.5)	314	8 (2.5)	25 (7.9)	0.5 (0.613)	5.4 (<0.001)
13	GDH/CDT only	1146	29 (2.5)	480 (43)	628	125 (19.9)	64 (12.7)	17.4 (<0.001)	−30.3 (<0.001)
14	GDH/CDT+PCR	443	34 (7.7)	24 (5.9)	921	15 (1.6)	101 (11.1)	−6.1 (<0.001)	5.2 (<0.001)
15	GDH/CDT only	525	0	45 (8.6)	543	0	79 (14.5)	0	5.9 (<0.001)
16	GDH/CDT+PCR	1095	101 (9.2)	136 (13.7)	918	28 (3.1)	91 (10.2)	−6.1 (<0.001)	−3.5 (<0.001)
17	GDH/CDT only	4391	385 (8.8)	837 (20.9)	4234	375 (8.9)	792 (20.5)	0.1 (0.884)	−0.4 (0.685)
18	GDH/CDT only	1638	709 (43.3)	113 (12.2)	1875	933 (49.8)	141 (15)	5.3 (<0.001)	2.8 (0.08)
Tertiary care hospitals		13563	1279 (9.4)	1029 (8.4)	11111	1440 (13)	919 (9.5)	3.6 (<0.001)	1.1 (0.004)
General hospital, >400 bed		7772	414 (5.3)	723 (9.8)	6953	899 (12.9)	617 (10.2)	7.6 (<0.001)	0.4 (0.482)
General hospital, <400 bed		1549	37 (2.4)	490 (32.4)	942	133 (14.1)	89 (11)	11.7 (<0.001)	−21.4 (<0.001)
HMO laboratories		8092	1229 (15.2)	1155 (16.8)	8491	1351 (15.9)	1024 (16.9)	0.7 (0.199)	0.1 (0.958)
PCR or GDH/CDT+PCR		14919	1680 (11.3)	1148 (8.7)	12247	2061 (16.8)	1224 (12)	5.5 (<0.001)	3.3 (<0.001)
GDH/CDT only		16057	1279 (8.0)	2249 (15.2)	15250	1762 (11.5)	1605 (11.9)	3.5 (<0.001)	−3.3 (<0.001)
Total		30976	2959 (9.5)	3397 (12.1)	27497	3823 (13.9)	2829 (11.94)	4.4 (<0.001)	−0.1 (0.543)

[a]First period, testing of *Clostridium difficile* toxin (CDT) by EIA method by all laboratories, second period, change to methods as described in second column.
[b]Proportion positive was calculated among the accepted samples.
[c]CDT PCR was implemented for all glutamate dehydrogenase antigen (GDH) -positive, CDT-negative samples in the last 3 months of the survey.
[d]GDH/CDT alone was used in the first 9 months of the second period, and CDT PCR was used in the following 3 months. For comparison, data from the first 9 months only are presented. In the 3 months of CDT PCR use, 125 samples were tested, of which 11 (8.8%) were positive.

Discussion

The past decade has been notable for dramatic changes in the epidemiology and morbidity of CDI in North America and other parts of the developed world [1]. At the same time, new diagnostic methods have become available, allowing improved sensitivity in CDI diagnosis. Consequently, the evaluation of CDI incidence, especially on a national level, has become problematic, as detailed data regarding the methodology used by each clinical laboratory are difficult to collect and interpret. In this study, we performed the first national survey evaluating the effect of methodological changes on the proportion of positive samples. Although a minority of the laboratories elected not to participate, we were able to collect data from almost all laboratories providing diagnostic services to community clinics and LTCFs, and to c.65% of the GH service beds. Through this study, we were able to represent the effects of progress in CDI diagnostic methods on the proportion positive in a large variety of different institutions and to highlight the pitfalls that can be expected.

In our survey we found that by the time of the onset of the study, only 3 of 21 laboratories (14%) were using CDT EIA alone for the diagnosis of CDI. This percentage is lower than that reported in Australia, where 60% were still using CDT EIA alone [8]. However, at the time of that survey (2009 to early 2010) [8], all of the laboratories in Israel were still using CDT EIA (the implementation of the new methods had started by late 2010, long before the publication of the official Israeli guidelines). Among the remaining 18 laboratories, nine were testing also for GDH antigen (group B) and nine were using CDT PCR alone or combined with GDH/CDT testing (group A), as recommended by Israeli and other national guidelines [5,6]. The reason for not performing confirmatory testing by CDT PCR in the case of equivocal results (e.g., GDH+/CDT−) was not solicited in this survey, but was likely to be either financial constraints or availability difficulties (personal communications).

Our results demonstrated two main factors associated with the proportion of positive samples. We found that laboratories using CDT PCR alone or following GDH screening (group A) had a higher proportion, compared with those using CDT EIA, either alone (during the first period) or combined with GDH (during the second period in group B). The results of the multivariable analysis suggest that this difference is due not to variation in the epidemiology between the periods, but rather to differences in testing methods. Although this finding suggests that CDT PCR testing is likely to improve the detection of CDI, one must bear in mind that with it comes the potential for over-diagnosis of CDI, due both to analytical false-positive results and testing of asymptomatic carriers of *C. difficile*.

Surprisingly, CDT EIA testing (by various assays) alone was associated with a higher proportion positive than was CDT

TABLE 3. Proportions of positive tests for *Clostridium difficile* using *C. difficile* toxin (CDT) EIA, GDH antigen and CDT PCR during the second period of the survey

Lab.[a]/ methods[b]	Non-rejected samples	GDH+/ CDT+ (%)	GDH+/ CDT− (%)	CDT PCR, positive/ tested (%)	CDT EIA sensitivity (%)[c]
1/A	2223	158 (7.1)	243 (10.9)	139/243 (57)	53
2/B	4187	232 (5.5)	527 (12.6)	4/11 (36)	NA
5/B[d]	889	31 (3.5)	19 (2.1)	NA	NA
6/A	1479	107 (7.2)	163 (11)	60/150 (40)	NA
8/A	205	18 (8.8)	11 (5.4)	8/11 (73)	69
9/B	1478	144 (9.7)	172 (11.6)	5/8 (62)	NA
10/A	476	47 (9.9)	50 (10.5)	33/50 (66)	59
12/B[e]	306	25 (8.1)	4 (1.3)	NA	NA
13/B	503	64 (12.7)	17 (3.4)	NA	NA
14/A	906	51 (5.6)	82 (9.1)	50/82 (61)	50
15/B	543	79 (4.5)	32 (5.9)	NA	NA
16/A	890	69 (7.8)	41 (4.6)	22/38 (58)	NA
17/B	3859	792 (20.5)	36 (0.9)	NA	NA
18/B	942	128 (13.6)	81 (8.6)	13/15 (87)	NA
Total	18886	1945 (10.3)	1478 (7.8)	334/608 (53)	NA

[a]The report excludes laboratories that used CDT PCR as their primary diagnostic method or were unable to provide a detailed report of the number of glutamate dehydrogenase antigen (GDH) -positive, CDT-negative samples.
[b]Methods: A, combined GDH/CDT testing, with CDT PCR performed for most cases (>90%) of GDH-positive, CDT-negative samples; B, combined GDH/CDT testing, with CDT PCR performed for the minority of GDH-positive, CDT-negative samples.
[c]Sensitvity of CDT EIA was calculated only for laboratories that performed CDT PCR in all GDH+/CDT− samples, using CDT PCR as comparator.
[d]Data regarding the number of GDH+/CDT− samples were available for 8 months only.
[e]GDH/CDT testing was performed for 9 of 12 months.

EIA performed in the context of the combined GDH/CDT C. Diff Quik Chek Complete lateral flow assay (TECHLAB®). Indeed, the average sensitivity (compared with CDT PCR) was only 59% with a range of 50–75%. These results are similar to previous studies in which the sensitivity values of CDT EIA were 48–78% [14–17]. They are also consistent with many studies that found variable sensitivity and specificity values in different EIA [4]. Due to the nature and design of our study, it was difficult to accurately compare different CDT EIA in use by different laboratories. Our results do, however, highlight two important messages: (i) it is critical to validate any new assay, including commercial PCR assays, with the 'gold standard' method, either toxigenic culture or cell culture cytotoxicity neutralization assay, as the analytical parameters of similar methods may differ significantly, and (ii) in laboratories that are shifting from CDT EIA to GDH-based testing for CDI, it is essential to perform confirmatory CDT testing by methods other than EIA alone (e.g., CDT PCR), because of the low sensitivity of EIA and the variable proportions of toxigenic versus non-toxigenic strains in different populations.

Overall, the proportion of samples rejected increased during the second period, and the extent of the change was particularly high in laboratories that applied or added rejection criteria in the second period. In contrast, the proportion of rejected samples decreased in the two laboratories that did not apply any criteria. Surprisingly, HMO-affiliated laboratories also had a high proportion of positive samples. Considering that direct communication between clinicians and laboratories, and therefore appropriate use of laboratory tests, is not expected to be more common in the community than in the GH, this difference probably reflects an actual difference in epidemiology, e.g. the LTCF versus the GH population. Regrettably, we could not differentiate between tests originating from community patients and those from LTCFs to further explore the source of this difference. Other effects of the change in rejection policy on the proportion positive varied in the different groups. Of note, in laboratory no. 13, we initially observed a very high proportion of positive samples, most likely through the lack of any rejection policy. The proportion decreased following the application of rejection criteria (Table 2, Fig. 1). These changes are indicative of the tremendous effect that diagnostic strategies, both pre-analytical and analytical, may exert on the reported incidence, and thereby on influence surveillance, both within and among medical facilities.

This study has a number of limitations, inherent to its design. First, it was not designed to provide a head-to-head methodological comparison nor an accurate estimation of CDI incidence. Second, the inter-laboratory variability in the study dates complicates the evaluation of possible regional epidemiological changes (e.g. the emergence of an epidemic strain) that might have had an effect on the proportion of positive tests. Finally, our inability to distinguish between community-onset and LTCF-related cases diagnosed at the HMO laboratories, prevent us from discerning differences related to these two very different populations, and therefore limit our ability to compare the results of the HMO and the GH laboratories. Despite these limitations, our study met its objectives, and it provides unique, valuable data from a national perspective regarding recent advances in CDI diagnosis. It highlights that in addition to comparative methodological studies, national surveys are necessary to improve the standardized application of new methods and to evaluate their effects in real-life conditions.

Transparency Declaration

Nothing to declare.

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